Robust Summary - Polyethylbenzene Bottoms Stream

Toxicity to Aquatic Plants (e.g., algae)

Test Substance:	Polytheylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN. 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/Guideline:	OECD Method 20 1
Type (test type):	static, water accommodated fractions in sealed vessels
GLP:	yes
Year (study performed):	2005
Species/strain no. and source:	***Pseudokirchneriella subcapitata*** obtained from University of Texas
Element Basis:	area under the growth curve, growth rate
Exposure Period:	72 hours
Analytical Monitoring:	yes
Statistical Methods:	EC values determined using a logistic model; NOEC values determined using one-way ANOVA with Dunnett's test
Test Conditions: Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organism supplier, age, size, loading.	Exposure solutions of PEB Blend were prepared as water accommodated fractions (WAF) in freshwater algal nutrient medium. The medium was prepared according to guideline procedures and supplemented with $NaHCO_3$ (500 mg/L) by adding quantities of reagent grade salts to purified and sterilized water. After adding the salts, the medium was pH-adjusted to 7.5 ± 0.1 using 0.1 N HCl. WAF solutions were prepared by adding 0. 10-mL volumes of PEB Blend standards made in acetone to 2.0 L of medium in a 2.0-L glass aspirator bottle. Each aspirator bottle was sealed and stirred for approximately 1.8 hours. Stirring was adjusted to create a vortex of no greater than 25% of the height of the solution in the bottle. After stirring, the solutions were allowed to settle for 40 minutes. The aqueous phase was drawn from the bottom of each aspirator bottle into nine replicate test flasks. WAF solutions were created in this manner for PEB Blend loading rates of 65, 130, 250, 500, and 1000 µg/L. A negative control and a vehicle control with acetone at 0.05mL/L were prepared in a similar fashion. The 130mg/L treatment contained an additional replicate that served as an abiotic control group. This replicate was not inoculated with algae. Replicate flasks consisted of 125-mL Erlenmeyer flasks with Teflon'-lined screw caps. When completely filled and sealed with no headspace, flasks held approximately 147 mL of test solution. Replicates were filled and sealed in this manner to minimize potential loss of volatile components in the test substance. The freshwater alga, ***Pseudokirchneriella subcapitata,*** was maintained in the laboratory in liquid cultures. The origin of the culture was the Department of Botany, Culture Collection of Algae, University of Texas at Austin. New cultures were periodically cloned from the existing culture derived from the parent stock. The culture used in this test was seven days old at test initiation. The test commenced when the flasks were filled, inoculated with algae to a starting density of approximately 1.4 x 1 0^4 cells/ml, sealed, and randomly

placed on a rotary shaker set a approximately 100 rpm. Flasks were incubated at 24 ± 2°C for 72 hours under continuous lighting. Lighting was produced by cool-white fluorescent bulbs at an intensity of 8,600 ± 10% lux. Temperature and light intensity were monitored throughout the study. Cell densities were determined using a light microscope and a haemacytometer at 24, 48, and 72 hours. At each counting period, three replicate flasks were destructively sampled and counts were made of the cell densities in each replicate flask. At the beginning of the test, measurements of pH were made in samples taken from the aspirator bottle of each treatment. At the end of the test, pH of the solution in the first replicate of each treatment was measured. The temperature of the testing area was measured continuously during the test.

The pH of the test solutions ranged from 7.9 to 8.0 at test initiation and from 8.0 to 9.4 at 72 hours. Temperature of the test solutions ranged from 22.6°C to 23.0°C when measured at 0 and 72 hours. The continuous temperature recording of the testing area ranged from 23.7°C to 24.4°C.

Concentrations of PEB Blend in the WAF solutions were measured by gas chromatography in samples from each treatment level at the beginning and end of the test. Measurements were based on a validated method that summed the responses from six marker peaks within the PEB Blend chromatogram. Concentrations were determined directly from a standard curve.

The area under the curve, and growth rate were taken as indices of algal growth and were calculated for each treatment using cell densities determined at 24, 48, and 72 hours.

Area Under the Growth Curve (AUGC):

$$A = (N_1 - N_0/2) \times t, + (((N_1+N_2 - 2N_0)/2) \times (t_2 - t_1))) + \ldots + (((N_{n-1} + N_n - 2N_0)/2) - t_{n-1}))$$

A= area under the growth curve
N_0 = Nominal number of cells at t_0
N_1 = Mean cell density at t_1
N_2 = Mean cell density at t_2
N_n = Mean cell density at t_n
t_1 = time of first measurment (hours from start)
t_2 = time of first measurment (hours from start)
t_n = time of nth measurement (hours from start)

Growth Rate:

$$\mu = - \ln N_0)/(t_n - t_0))$$

μ = average specific growth rate
N_0 = Nominal cell density at t_0
N_n = Measured dell density at t_n
t_0 = Time of beginning of test (hours)
t_n = Time after beginning of test (hours)

The response of the negative and vehicle control groups was assessed to determine whether or not they could be pooled by comparing the 72-hour means for the area under the growth curve and growth rate. Tests for normality and homogeneity of variance were performed along with a t-test between the two control groups. The analyses showed a statistical difference between the control groups for biomass and growth rate; therefore, the vehicle control response was used for the calculation of inhibition values for the treatment group's responses.

	Calculation of Inhibition: Percentage inhibition of growth (I_A) and growth rate (I,) were calculated by the following equation: Inhibition, % = (vehicle control mean – treatment mean) / vehicle control mean x 100
Results: Nominal Loading Rate Conc., µg/L Mean Measured Conc., µg/L Element Values	0 (control), 0 (vehicle control), 65, 130, 250, 500, and 1000 µg/L 0 (control), 0 (vehicle control), 44.4, 95.9, 192, 401, and 69 1 µg/L 72-h E&50 = 320 µg/L (95% CL = 310 and 330 µg/L) (nominal loading rate) 72-h E_rC50 = 640 µg/L (95% CL = 610 and 680 µg/L) (nominal loading rate) 72-h NOEC = 130 µg/L (nominal loading rate) 72-h E_bC50 = 251 µg/L (95% CL = 241 and 261 µg/L) (mean measured concentration) 72-h E_rC50 = 485 µg/L (95% CL = 463 and 507 µg/L) (mean measured concentration) 72-h NOEC = 95.9 µg/L (mean measured concentration) Negative and vehicle control responses over the 72-h period exceeded the minimum acceptable increase in cell density as specified in the guideline.
Conclusion: (Laboratory Contractor)	The 72-hour NOEC was the nominal loading rate of 130 µg/L or the mean measured concentration of 95.9 µg/L, based on a lack of statistically significant reduction of biomass and growth rate at or below this test substance treatment. Based on biomass, the 72-hour E_bC_{50} was the nominal loading rate of 320 µg/L or the mean measured concentration of 251 µg/L. Based on growth rate, the 72-hour E_rC_{50} was the nominal loading rate of 640 µg/L or the mean measured concentration of 485 µg/L.
Reliability:	**1.** Reliable without restrictions.
Reference:	Hicks, Stephen L. 2006. Toxicity of a Polyethylbenzene Bottoms Stream Blend (PEB Blend) to the Unicellular Green Alga, ***Psudokirchneriella subcapitata***. ABC Laboratories, Inc., Columbia, MO. Sponsor: American Chemistry Council, Arlington, VA
Other (source) Last changed	